TOWARDS TUMOR THERAPY WITH INTERFERONS, Part II. Interferons: In Vivo Effects

By Mathilde Krim

PHARMACOLOGY AND TOXICOLOGY

THE PHARMACOLOGY of interferons has been studied with human leukocyte interferon and with mouse and rabbit interferons. These have a short half-life in the blood, irrespective of the route of administration, though the most rapid clearance follows the intravenous route; intramuscular injections result in peak blood levels after 2–4 hr, lasting for 4–6 hr. With intramuscular doses of $2 \times 10^5$ IU/kg body weight in man, one can maintain about 100 U of interferon activity/ml of plasma for 12 hr. The plateau phase can be prolonged with a higher initial dose. This slow clearance phase is believed to be due in part to the release of previously tissue-bound interferon. Binding and subsequent elution have been documented for cultured cells.

Only a fraction of the interferon administered intramuscularly can reach the respiratory tract, the cerebrospinal fluid, the eye fluids, and the brain, although these compartments may contain high titer endogenous interferon when they harbor viral infections. There is minimal urinary or intestinal excretion. When lactating mice are treated with interferon inducers, some interferon can be found in the milk and it appears to protect suckling animals. This is a unique instance of administration effective per os.

The pharmacology of human fibroblast interferon is less well known, and it has recently become obvious that it is quite different from that of leukocyte interferon. In vitro, fibroblast interferon has all the properties of an interferon, but it has, so far, been less effective therapeutically. Clearance is more rapid, and it may not be detectable at all in the circulation following i.m. administration. It appears very sensitive to inactivating factor(s) present in body fluids and/or extracts of muscle tissue. It may actually have desirable properties, having been particularly effective in slowing down the rate of multiplication of osteogenic sarcoma cells in vitro.

Mouse interferon is also rapidly cleared from blood and inactivated by muscle extracts. Moreover, when mouse "leukocyte" interferon (produced by mouse spleen cells) was compared to the mouse "fibroblast" (L-cell) interferon, their major molecular species were found identical physicochemically, biologically, and autogenically. Thus, the mouse seems to lack an equivalent of human leukocyte interferon. Mouse interferon also seems less effective as an antitumor agent than human leukocyte interferon (it does not induce tumor regressions), perhaps because, like human fibroblast interferon, it is rapidly inactivated in vivo.

Human leukocyte interferon preparations with a specific activity of $\geq 10^6$ IU/mg protein (0.1%–1% interferon protein) are well tolerated when given subcutaneously, intramuscularly, or even when injected intravenously or intracisternally. Side effects have consisted of dose-related febrile responses, usually more marked following the first few injections, whatever the route of administration. Transient myalgia and chills following each injection have been reported with daily doses greater than $1.7 \times 10^5$ IU/kg body weight. Fatigue and malaise are also cause for complaints, particularly by older or debilitated patients.

The use of more purified preparations ($\geq 10^7$ IU/mg...
protein, 1%-10% interferon protein) has resulted in a significant decrease in the severity of adverse reactions and allowed the daily administration of $5 \times 10^7$ IU/kg body weight.\textsuperscript{14-16} However, at higher daily doses, other effects have been observed that may result from the interferon’s intrinsic biologic activities. Among them are a decrease, and stabilization at a lower level, of leukocyte, platelet, and reticulocyte counts and, in a proportion of patients, some hair loss and diarrhea. These effects, perhaps due to a slowing down of the rate of cell multiplication, have been mild, completely reversible within days of the cessation of treatment, and apparently without adverse consequences.\textsuperscript{14} Fever, which is caused by preparations of any degree of purity, may be due to interferon-induced production of histamine and prostaglandin E.

Attempts have been made to reach toxic doses in mice. Interferon was first administered daily, starting at birth, and for a period of 1 yr, at doses comparable to those given to adult animals in effective antitumor treatment. The animals showed normal growth rate and normal development.\textsuperscript{17} However, newborn mice injected daily with 50,000 U (100-fold the present clinical dosage), failed to gain weight and died during the second week of life with extensive liver degeneration. If treatment was stopped after 1 wk, mice survived, only to die 1 mo later with severe glomerulonephritis.\textsuperscript{18,19} Endogenous interferon may have similar effects: very young mice infected with lymphocytic choriomeningitis virus develop very high titers of endogenous interferon and die of glomerulonephritis. Not only do they survive when given antiinterferon globulin, but the glomerulonephritis does not develop, pointing to endogenous interferon, and not to virus, as the possible cause of the kidney disease.\textsuperscript{20}

These experiments suggest that interferons may have age-related adverse effects. Further careful preclinical and clinical toxicologic studies are needed since interferon treatment for cancer is likely to require relatively high doses over long periods. In children such treatment could have profound effects on immune functions, cell multiplication rates, cell differentiation, and other cellular functions.

**PROPHYLACTIC AND THERAPEUTIC EFFECTS OF INTERFERRONS**

**Interferons as Antiviral Agents**

**In Animals**

The scarcity and low titer of early interferon preparations, as well as a less sophisticated knowledge of viral pathogenesis and the mechanisms of interferon antiviral action, account for a number of early negative or inconclusive studies. Nevertheless, once it was realized that dose and time of interferon administration must be related to kind and amount of challenge virus,\textsuperscript{21-27} it has become clear that both prophylaxis and therapy of viral infections can be achieved for both cytopathic and oncogenic, RNA- and DNA-containing, viruses. These include Sindbis and Semliki Forest virus, two togaviruses; the rhabdoviruses, vesicular stomatitis virus and rabies virus; a picornavirus, encephalomyocarditis virus; vaccinia virus; the oncornaviruses (Gross, Friend, Rauscher, radiogenic leukemia, Harvey strain of the murine sarcoma, Rous sarcoma, and the DNA tumor virus, polyoma virus.\textsuperscript{24-26} On the other hand, administration of antime use interferon globulin greatly enhances the severity of experimental and natural infections by both cytopathic and oncogenic viruses.\textsuperscript{27,28}

**In Man**

Clinical investigations with all interferons have been severely limited by the paucity of appropriate preparations. For this reason, interferon was first used topically. As early as 1962, a very small amount of interferon, produced by cultured monkey cells (primate interferons are active in human cells), inhibited "takes" in volunteers vaccinated against smallpox,\textsuperscript{29} and vaccinal keratitis was treated effectively in man\textsuperscript{30} as in rabbits.\textsuperscript{31} Human interferon was later used against myxoviruses and herpes simplex infection, applied topically to the respiratory mucosa\textsuperscript{32} and the corneal epithelium.\textsuperscript{33,34} Unequivocal antiviral effects were demonstrated in each trial.

A role for systemic interferon in protection from lethal rabies infection can be predicted from animal studies\textsuperscript{35-37} but the experiment has not, as yet, been done in man since fatal central nervous system infection develops only in a proportion of those bitten by rabid animals. There is evidence for systemic interferon-mediated protection against viral infections in interferon-treated cancer patients. Cancer patients are notoriously prone to viral diseases particularly during and after immunosuppressive therapies. Patients receiving interferon as experimental antitumor therapy may be protected from viral infection while under treatment.\textsuperscript{4,38-40} As for antiviral therapy, systemic administration of interferon has been tried so far in chronic active hepatitis B and in herpes zoster in adults, and in varicella and cytomegalovirus infections in children.

**Chronic active hepatitis B** was selected for trials because several viral markers can be quantitated in patients’ sera. (The markers are a viral surface antigen, HbAg, a viral core antigen, HbAg, the viral enzyme DNA-polymerase, a viral double-stranded
DNA and a soluble antigen associated with transmission of the disease. Usually, patients have also a high antiserum titer against the Hb antigen and some have circulating Dane particles, believed to be the viral etiologic agent.) In one trial involving patients who had high circulating levels of virus markers for more than 6 consecutive months, each injection of interferon elicited a dose-related response, particularly for DNA-polymerase levels. Other markers decreased only with repeated daily injections. If interferon was given for 10 days or less, these effects were transient; they became longer-lasting upon more prolonged treatment and could persist for several months beyond the treatment period. Early, intensive high-dose interferon therapy (≥ 10⁶ U daily), followed by maintenance therapy at low dose (10⁶ U daily), has now resulted in suppression of all virus markers and a progressive improvement in liver function. Two other studies, one in man with fibroblast interferon and the other in chimpanzees with the inducer PICL, have confirmed the sensitivity of Dane particle markers to interferons. Hepatitis B is one of the many viral diseases for which there is at present no effective therapy. Chronic cases arise in about 10% of those suffering at any one time from acute hepatitis, and those with the chronic active form are not only carriers, but contagious. The disease is on the increase in the western world and can affect as many as 20% of the population of developing countries. An antiviral treatment that can suppress the infectivity of hepatitis virus carriers also offers hope that the disease itself may one day be eradicated.

Controlled trials were carried out with interferon in 90 cancer patients with herpes zoster (shingles). Endogenous interferon may play an important role in the natural defense against the varicella zoster virus whose reactivation causes shingles. Patients whose vesicle fluid has a high endogenous interferon titer more rarely develop disseminated disease, and heal more rapidly. These titers appear to have important prognostic value. Prospective, randomized trials in herpes zoster have established the therapeutic effect of interferon; acute herpetic neuralgia subsided faster, cutaneous dissemination of lesions was lessened, visceral complications and the resulting mortality in cancer patients were reduced.

Interferon is also being tested for prevention of herpes virus reactivation following surgery for trigeminal neuralgia. There appears to be a decrease in frequency of virus reactivation and isolation following surgery. Encouraging preliminary results have also been reported in a study of chickenpox in leukemic children.

Cytomegalovirus (CMV) infections of the newborn have been treated experimentally with interferon. Excretion of the virus in urine was suppressed. Presently, it seems clear that congenital CMV infection can be controlled by interferon.

CMV is of major importance in transplantation medicine; renal transplant patients often succumb to CMV-induced hepatitis, pneumonitis, and/or mononucleosis. CMV induces immunosuppression and leukopenia, which may result in bacterial and fungal infections. Double-blind, placebo-controlled trials have been conducted with both fibroblast and leukocyte interferons in renal transplant recipients. While fibroblast interferon failed to prevent viral infections, the incidence of virus excretion, and viremia with CMV, herpes simplex, and Epstein Barr viruses were significantly decreased in patients treated with leukocyte interferon.

Interferons as Inhibitors of Intracellular Parasites

Few studies have been on interferon effects on the growth cycle of intracellular parasites. In cell cultures and in eggs, interferon can inhibit Chlamydiae which cause infections such as psittacosis in man and are closely related to the agent of trachoma.

Both interferon and interferon-inducers have been effective in inhibiting murine infection by sporozoites of Plasmodium berghei. Effects on human infection by plasmodia have yet to be reported.

Interferons as Antitumor Agents

Antitumor Effects in Animals

Interferon preparations of different kinds and degrees of purity can exert antitumor effects.

Experimental, virus-induced tumors. Friend leukemia, Rauscher leukemia, and Moloney (Harvey strain) sarcoma of the mouse; Shope fibroma of the rabbit; Polyoma virus-induced tumors in the hamster; Rous sarcoma in the chicken; Herpes virus (Saimiri) induced lymphomas in the marmoset and the owl monkey, were all found susceptible to inhibition by interferon. A few early negative findings were reported. One instance of tumor enhancement following a single very high dose of interferon prior to tumor-virus inoculation may be attributed to the immunosuppressive effect of interferon on the primary immune response to the virus. With murine leukemia viruses interferon treatment has delayed or retarded tumor appearance and growth, resulting in prolonged

* Poly r(T) – Poly r(C), complexed with poly-L-lysine, stabilized in carboxymethyl cellulose.

† With human leukocyte interferon.
survival, but only when given after virus inoculation, and repeatedly over prolonged periods of time.\textsuperscript{55,56,57}

\textit{Spontaneous tumors.} AKR leukemia,\textsuperscript{58-60} and spontaneous mammary carcinoma\textsuperscript{61,62} of the mouse have been treated with interferon. Endogenous type-C (Gross) and type-B (Bittner) viruses are, respectively, involved in these diseases, and virions are produced continuously in large numbers by the tumor cells. The AKR leukemia is used extensively to evaluate antileukemic immuno- and chemotherapy because the system has been predictive of effectiveness in man.\textsuperscript{63} As for the experimentally-induced leukemias, interferon must be given to AKR mice daily, at relatively high doses (1–2 \times 10^4 U), over long periods, to obtain an effect. Treatment started in preleukemic AKR mice and continued for 1 yr, prolonged survival by about 100 days, and the overall incidence of leukemia was reduced from 95\% in control mice to 63\% in interferon-treated animals.\textsuperscript{58} When started at 6–8 mo of age, late in the preleukemic stage, treatment still achieved a delay in tumor appearance.\textsuperscript{60} With “very high” doses, i.e., \(\geq 10^7\) U/day, one investigator reported regressions of early disease.\textsuperscript{59} Although true regressions were not confirmed, at least a doubling of lifespan could be obtained with high doses even when treatment was started after clinical diagnosis,\textsuperscript{60} an effect which cannot regularly be achieved with many chemotherapeutic agents.\textsuperscript{63} It represents an antitumor effect obtained by some 0.1 \(\mu\)g interferon protein per day on a tumor load estimated to be some 10^9 cells. At present, interferon appears to be the most active antitumor agent in the AKR mouse tumor model.\textsuperscript{56}

Delayed and retarded tumor growth was obtained in the spontaneous mammary carcinoma of the mouse, with a modest increase in lifespan.\textsuperscript{61,62} No decrease in “gs” protein was observed, but since interferon inhibits oncornavirus assembly or release but not protein synthesis, this may not be surprising.

Worth mentioning along with these studies on “spontaneous” tumors is the finding that interferon-induced inhibition of both graft rejection and graft-versus-host disease in mice is associated with complete suppression both of the induction of oncornaviruses\textsuperscript{64} and of the high incidence of lymphoma\textsuperscript{65} that otherwise follow these conditions.

\textit{Transplantable tumors.} Although C-type oncornavirus particles can occasionally be seen in transplantable tumor cells—as in all mouse tumor cells—virus seems to play no part in their ability to multiply rapidly and to form either solid or ascites tumors in syngeneic or allogeneic hosts. Several transplantable mouse tumors (EA, EL4, L1210, RC19) have been studied for susceptibility to interferon in both kinds of hosts.\textsuperscript{56,66,68} Each tumor is highly malignant; with EA cells, for example, the LD50 corresponds to one cell. Even so, the antitumor effect of interferon can be marked. Following inoculation of 10^4 EA cells, and standard treatment with 2 \times 10^4 U of interferon daily for several weeks, 90\% of those mice treated survived more than 6 mo, while mortality was 100\% at day 22 in untreated animals. In Lewis lung carcinoma of the mouse, a metastasizing tumor usually highly resistant to therapy, interferon inhibited the growth of both primary tumors and lung metastases.\textsuperscript{57}

Other transplantable mouse tumors found susceptible to inhibition by interferon, were: Ehrlich carcinoma, sarcoma 180, Polyoma-induced sarcoma, Friend leukemia cells, and osteosarcoma. In the latter, interferon is active against both primary tumors and lung metastases.\textsuperscript{60-71} Negative results were reported with L929 cells transplanted in the mouse.\textsuperscript{72} However, treatment was with only 1000 U given 3 times per week. In other species, the Brown Pearce carcinoma of the rabbit and the Walker carcinoma of the rat appear sensitive to interferon.\textsuperscript{56}

Very little has been done with Type II mouse interferon, although it has been reported a more powerful antitumor agent than Type I. Given daily, 300 U of Type II injected directly into sarcoma MC-36 tumors, or 600 U injected systemically into mice with metastatic osteosarcoma\textsuperscript{70,71} have as much tumor-suppressive activity as \(\geq 10^4\) daily units of Type I interferon. However, Type II interferon preparations are rich in various lymphokines, and the antitumor activity cannot yet be attributed specifically to Type II interferon.\textsuperscript{73,74}

Malignant cells of human origin, i.e., HeLa, bladder cancer, mammary carcinoma, melanoma, and osteosarcoma cells, transplanted into nude mice have been reported susceptible to inhibition by \textit{human}, not mouse, interferon.\textsuperscript{75}

\textit{Chemically induced tumors.} Interferon treatment has inhibited tumor induction by 3-methylcholanthrene in the mouse; tumor development was either delayed\textsuperscript{76} or completely inhibited.\textsuperscript{77}

\textit{Radiation-induced tumors.} Low-dose, repeated treatment with interferon, started immediately after x-irradiation, significantly decreased the incidence of radiogenic lymphoma in the mouse.\textsuperscript{78} Since radiation induced an endogenous type-C virus, this may reflect an antiviral effect.

\textit{Combination antitumor therapies in animals.} Synergistic effects have been reported following interferon therapy in combination with various cytoreduc-
IN VIVO EFFECTS OF INTERFERONS

879

deitative methods. More than 70% of mice inoculated with LSTRA leukemia cells, and treated sequentially with BCNU (1.3, bis [2-chloroethyl]-1-nitrosourea) and interferon were cured, or became long-term survivors; interferon alone had no measurable effect, and BCNU alone produced only 25% long-term survivors. Changing the order of the combined treatment abrogated the effect. Among long-term survivors sacrificed at 90 days, only the animals treated with both BCNU and interferon were without residual leukemic cells. The treatment of overt AKR leukemia with cyclophosphamide, vincristine, or cytoxan followed by interferon has also resulted in more prolonged survival than either chemotherapy or interferon alone. Comparable results were obtained in a solid tumor, a transplanted mammary carcinoma, with the combination of adriamycin and interferon. Surgical resection of a primary mouse mammary carcinoma increased lifespan by 79%. With surgery followed by interferon, lifespan was increased by 215%. When the mild immunostimulant, isoprinosine (ineffective alone on transplanted sarcoma 180 cells), was given before interferon, a doubling of the number of long-term survivors could be obtained among sarcoma 180-bearing mice.

Whatever the mechanism, the antitumor effects of mouse interferon preparations can be attributed to the interferon itself because:

1) A dose–response correlation has been reported in several systems. Treatment with antiinterferon globulin has caused marked tumor enhancement in mice infected with Moloney sarcoma virus; tumors appeared earlier, were much larger, and killed animals more rapidly. In antiinterferon globulin treated mice, the tumor-inducing potential of Moloney sarcoma virus was increased 100-fold. The same was observed with Rauscher leukemia virus.

2) In a syngeneic system, presumed pure mouse interferon protein with an activity of 2.4 \times 10^9 IU/mg protein, has inhibited the \textit{in vivo} growth of a transplantable tumor to a similar extent, per antiviral unit, as partially purified or crude interferons.

Possible mechanisms of mouse interferon antitumor action. As is the case for its antiviral activity, the antitumor activity of interferon appears mediated by multiple mechanisms: (A) an antiviral effect, (B) direct effects on tumor cells, and (C) indirect, host-mediated effects. Which type of effect predominates depends on the system studied.

Suppression of the appearance of tumors induced by DNA-containing oncogenic viruses (involving nonproductive transforming cell-virus interactions) requires that interferon be administered prior to, or simulta-

neously with, the viral inoculum. The inhibitory mechanism is clearly an antiviral one, and must involve a block of an early event required for viral cell transformation, as has been observed \textit{in vitro}.

In the case of oncornavirus-induced tumors—whether experimental or spontaneous—large numbers of infectious and transforming virions are released during repeated cycles of virus multiplication, throughout the animal’s lifespan. Interferon does not inhibit oncornavirus translation, but their assembly or release. Not surprisingly, the suppression of such tumors requires that interferon be administered at high daily doses over long periods of time. Reduced or suppressed viremia is clearly the major component of the antitumor effect. In Friend leukemia, an inhibition of cell multiplication (a direct effect) and antibody-dependent cytotoxic immune reactions (a host-mediated effect) also appear to play a role.

In the case of transplantable tumors, which may be better models for human neoplastic disease, interferon may exert direct effects on tumor cells. Inhibition of tumor cell multiplication, changes in cell surface properties, and/or suppression of tumorigenic potential, have all been observed \textit{in vitro}. There is evidence, as well, for host-mediated mechanisms: L1210R cells, resistant to direct interferon action \textit{in vitro}, are susceptible to its antitumor effect \textit{in vivo}. Mechanisms underlying host-mediated antitumor effects may include interferon-enhanced cytotoxicity by sensitized T lymphocytes, activation of natural killer cells, and enhanced cytotoxicity by macrophages. All these effects are reported to occur both \textit{in vitro} and in mice.

Additional, more speculative mechanisms may also be at play: tumor cells secrete various factors (angiogenesis factor, plasminogen activator, and others). Secretion of plasminogen activator may be inhibited by interferon, and interferons may suppress other tumor cell products, or modify host cell sensitivities to them.

Antitumor Effects in Man

To date, almost all studies of systemic antitumor activity have been with human leukocyte interferon. Because it is scarce, human fibroblast interferon has been used mainly \textit{in vitro} or for injection into, or near, superficial tumor nodules. The evaluation of lymphoblast interferon has barely begun and Type II human interferon has not yet been produced on a sufficiently large scale for use \textit{in vivo}.

Daily injection, for 30 days, of 0.5 \times 10^6 U of human fibroblast interferon (produced at Roswell Park Memorial Hospital) into superficial tumor
nODULES OF MELANOMA, BREAST CARCINOMA, AND PROSTATE CANCER RESULTED IN A MARKED INHIBITION OF GROWTH IN ABOUT HALF THE TREATED TUMOR NODULES; SEVERAL WERE REDUCED TO 25% OF THEIR ORIGINAL SIZE WITHIN 2 WK. HISTOLOGY REVEALED HEAVY INFILTRATION OF ALL TREATED NODULES WITH LYMPHOCYTES, AND IN THE CASE OF MELANOMA, DECREASE OR DISAPPEARANCE OF TUMOR LYMPHOCYTES.\(^75,93\) A SINGLE PATIENT WITH HODGKIN’S DISEASE HAS BEEN TREATED SYSTEMICALLY WITH 8-10 \(\times\) 10⁶ U OF FIBROBLAST INTERFERON DAILY, WITH DISAPPEARANCE OF BONE INFILTRATION.\(^96\)

**Human leukocyte interferon** is presently being tested topically\(^95\) and systemically\(^75\) against several human tumors. These trials are in different clinical centers, with interferon from different sources but comparable in titer (10⁶-10⁷ IU/ml) and specific activity (10⁵-10⁶ IU/mg protein), all prepared by Cantell’s method. All trials have involved small numbers of patients, and the results summarized below concern only the earliest exploratory attempts at demonstrating a response on the part of various types of tumors, following systemic administration.

**Classical osteogenic sarcoma.** The first trial with interferon in cancer patients was started by H. Strander, in Sweden, in 1972, on this highly malignant tumor of the bone. Primary treatment is surgical, usually through disarticulation or amputation. Reports on “historical series” indicate that fatal multiple gross pulmonary metastases develop predictably in a majority of patients within 12 mo following diagnosis of the primary lesion.\(^96\) A proportion of those receiving only surgical treatment do not develop metastases and may have a normal lifespan. The proportion of long-term survivors (17% in historical groups) may have increased to 25%-30% in recent studies, due, perhaps, to a higher incidence of cases with larger primary tumors at the time of surgery in the historical series.

Although “historical series” have been used as controls for trials of potential chemotherapeutic drugs,\(^97\) a new series with “contemporary controls” receiving only surgery has been developed in Sweden for the interferon trial. Prognostic factors appear to be equally distributed between controls and interferon-plus surgery patients. (The only exception, more bone resections followed by reconstructive surgery, rather than amputation or disarticulation, have been done in the interferon group. If this difference is meaningful, it is likely to bias the results against the interferon-treated group.)

In this trial, still ongoing at the Karolinska Hospital, consecutive patients are included who present with (A) a primary tumor located in the long bones or the pelvis; (B) normal chest x-ray on admission; and (C) diagnosis of osteogenic sarcoma based on concurring histologic diagnosis by several pathologists. Treatment with human leukocyte interferon (produced by Dr. Kari Cantell), starts immediately upon diagnosis, prior to surgery, with 3 \(\times\) 10⁶ U i.m. per day and is continued daily for 1 mo. The same dose is then given 3 times weekly for 17 additional mo, and then treatment is stopped. No other therapy is given. Chest x-rays are performed at intervals of 2 mo for interferon-treated patients and 5 mo for the controls. Patients tolerate treatment very well and receive it either at home or on an ambulatory basis.\(^98,99\)

Strander has reported recently\(^75\) that 5 of 10 interferon-treated patients have remained alive (and disease free) after 5 yr, while among contemporary control patients, this is so for 5 of 21 patients. He has also reported that when interferon-treated patients develop metastases, these are often fewer in number than is usually observed in the natural course of the disease. This would, in itself, offer better opportunities for their surgical resection which, when possible, can constitute curative treatment.\(^100\) In addition, the average survival time between biopsy and death—in those patients of each group who developed metastatic recurrences—was reported to be 25 mo for interferon-treated patients, 20 mo for contemporary control patients, and 13 mo for the historical control patients.

No statistical analysis of these various sets of data were presented, and although strongly suggestive, they are still difficult to interpret. Continuing inclusion of cases in this ongoing trial, and long-term follow-up of more patients, will be necessary for a critical evaluation and interpretation of these data.

Another study in this disease, using lymphoblast interferon as adjuvant therapy, is underway in Austria. This study includes 12 mo of prophylactic chemotherapy with high-dose methotrexate. No results are yet available.\(^101\) In another trial, conducted by T. Kishida in Japan, three patients with osteogenic sarcoma metastatic to the lungs were treated with high doses of leukocyte interferon (\(\geq\) 10⁶ IU/day). Two showed temporary regression of pulmonary metastases.\(^75\)

**Laryngeal papilloma.** A group of 7 patients with recurrent laryngeal papilloma is being treated with leukocyte interferon by H. Strander, in Sweden. Histologically, virus-like inclusions are seen in the laryngeal epithelium of these benign tumors. All patients treated are children with long histories of repeated surgery; two have had tracheostomy for papillomas of the larynx. Tumor size can be followed by endoscopy, and biopsies can be obtained. Cantell leukocyte interferon is given i.m., 3 \(\times\) 10⁶ U, either once, twice, or three times weekly, with no other treatment. In all seven patients, complete regressions
occurred gradually over a period of several months. Relapses were noticed within 2 mo of cessation of treatment. Regression and relapse have been followed several times on each patient. Two patients are presently tumor-free on a biweekly schedule of interferon administration; one patient has had no recurrence during nearly 1 yr without treatment.175

**Bladder papilloma.** A small group of cancer patients were treated with leukocyte interferon in Denmark.102 Three patients had had, for several years, multiple bladder papillomas of grades II and III. These lesions regressed on interferon therapy (4 x 10^6 U, i.m., 3–7 times weekly). Complete regressions were achieved after 2–17 mo of treatment.175

**Multiple myeloma.** Several groups have undertaken clinical trials following early encouraging results in this disease.103 In a Karolinska study, patients receive leukocyte interferon as sole therapy. Standard treatment is 3 x 10^6 U of leukocyte interferon i.m. daily. A preliminary report indicates that among 12 patients with no prior therapy, 4 with advanced disease when treatment was started responded neither to interferon nor to subsequent chemotherapy. Among the 8 patients with less advanced disease 1 showed no change, 4 had partial remissions (50% decrease in paraprotein levels and/or Bence-Jones protein). In one of these there was a complete regression, within 7 wk, of a large cranial soft tumor. Three patients showed complete remissions (all disease parameters normalized). Among four additional interferon-treated patients who had received prior chemotherapy and were resistant, one had a clear partial remission.

Starting in February 1978, J. Gutterman (M.D. Anderson Hospital, Houston) has treated 10 myeloma patients with Cantell leukocyte interferon. Most were treated for 8 wk with 3 x 10^6 U of interferon, given daily, i.m. Those who achieved an antitumor response were maintained on a 3 x per week schedule at the same dose. Some patients were on higher doses, i.e., 9 x 10^6 daily.14,75,104

Positive response to treatment was defined as in the Karolinska study. A positive response was obtained in six of the ten patients. Among four previous responders to chemotherapy, three responded to interferon, including one with complete remission; among three patients who had not responded to chemotherapy, one responded to interferon. No patient with very large tumor masses responded, while there were five responders among six patients with intermediate tumor masses. Responsiveness to interferon despite resistance to chemotherapy was observed in three cases, and these patients were subsequently maintained on a low-dose interferon regimen for several months. A scalp lesion began to regress 2 wk after the start of interferon therapy, and disappeared. In three responding patients, there was a return of immunoglobulins IgA and IgM to normal concentrations.

**Leukemias.** Small numbers of patients with acute and chronic leukemias have received interferon with suggestive evidence of response14,75,104,107,108. Controlled prospective studies have yet to be undertaken.

**Metastatic breast cancer.** Little work has been done so far in patients with solid tumors. The first extensive studies reported have been by Gutterman and coworkers on 17 patients with breast cancer.14,75,104,109 Patients were selected who had superficial metastases, which allowed measurement of tumor size. Most had been heavily treated previously, and many had failed to respond to conventional therapy. Two doses were used, either 3 x 10^6 U daily, or 9 x 10^6 U daily. Five of twelve women receiving the lower dose, and two of six receiving the higher dose, responded. Regressions were observed in soft tissues, in bone metastases, and in marrow infiltrations. Some individual lesions cleared completely. Carcinoembryonic antigen (CEA) levels decreased in responding patients and platelets counts went up. Interferon-induced regressions of metastatic breast carcinomas have since been obtained also by others.109 In the total series of 38 patients treated by Gutterman, interferon treatment was accompanied by lowered white cell counts (median of 2600 mm), and a depression in both granulocytes and lymphocytes. There was no suppression of delayed hypersensitivity, but a transiently decreased response of lymphocytes to mitogens. No viral infections were observed. Side effects were less when interferon preparations with specific activity of ≥10^6 U/mg protein were used. Fever and fatigue were minimal and were clearly dose- and age-related. Minimal weight and hair loss has occurred in some patients.

**CONCLUSIONS**

The state of the art regarding the therapeutic use of interferon preparations can now be summarized as follows: prophylactic and therapeutic antiviral effects have been demonstrated in both animals and man, suggesting a significant potential role for interferon in the treatment of several common and serious human viral infections.

**Antitumor effects in animals,** mainly the mouse, have been obtained with interferon alone. Effects have been more pronounced with low tumor loads, or with combination of interferon and cytoreductive methods. Complete suppression of the appearance of "spontaneous" and experimental tumors was obtained in animals that were subsequently capable of a normal
lifespan. However, regression of gross, palpable tumors, has not been achieved in the mouse.

Antitumor effects in man, frequently including marked tumor regressions, have been reported for several types of tumors, both benign and malignant, in optimum regimen, particularly with regard to duration of treatment, has not as yet been defined.

Nevertheless, in view of the substantial proportion of responders among the patients treated so far, many of whom had advanced disease, and the still highly impure state of the preparations used (0.1%–1% interferon protein content), the results to date are strongly suggestive of therapeutic effectiveness. Controlled, expanded trials in various neoplastic diseases must now be undertaken.

Existing methods of interferon production in cell culture systems are adequate to support substantial further clinical studies, including phase III trials. However, the development of truly efficient and economical mass production methods depends entirely on concerted basic research and development efforts aiming at in vitro synthesis of interferons and/or exploitation of recombinant DNA technology for their production by prokaryotes or suitable eukaryotic cells. Since the scientific and technologic knowledge exists to pursue both approaches, the widespread use of interferon therapy under economically feasible conditions is likely to be possible in the future.

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M Krim